IN THE SPECIFICATION:

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This application is a divisional of U.S. Application No. 09/719,961 filed 24 September 2001, which is a U.S. National Stage filing of International Application No. PCT/NL00/00253 filed 19 April 2000, which claims priority to EP Application No. 99201204.7 filed 19 April 1999. International Application No. PCT/NL00/00253 additionally claims priority to U.S. Application No. 60/176,924. All of the foregoing applications are hereby incorporated herein by reference in their entirety.

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The amino acid sequence of one C-type lectin that was found to be involved in the binding of the dendritic cells to the T-cells is shown in SEQ ID NO: 1 and Figure 9. This C-type lectin receptor is essentially similar to the C-type lectin gp120 receptor described by Curtis et al. in Proc. Natl. Acad. Sci. USA, 89 (1992), p. 8356-8360 and in WO 93/01820. In particular, it has a high degree of homology (> 98%) to the amino acid sequence given in SEQ ID NO: 1 of WO 93/01820. It is a group II C-type lectin of 404 amino acids; with an apparent Mr of about 44 kDa; and with a first domain (Met 1 to Ala 76) comprising a cytoplasmic amino terminus, a second domain (Ile 77 to Val 249) comprising tandem repeats, and a third domain (Cys 253 to Ala 404) with a high degree of homology to other known C-type lectins which are type II membrane proteins. Further characterization is given below.

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Thus, the monoclonals of the invention constitute a very useful diagnostic and research tool, for use both *in vitro* as well as *in vivo*. Possible non-limiting fields of application include the study of dendritic cells and their function and interactions; the study of the immune system; the detection of dendritic cells and/or C-type lectins in cells, tissues or biological fluids such as synovial tissue and skin tissue/skin cells (dermal dendritic cells); as well as the study of the role dendritic cells play in biological processes or disease mechanisms, such as cancer (as dendritic cells are exploited *in vivo* in clinical trials to eradicate tumor formation and development) and auto-immune diseases (including for instance rheumatoid arthritis).

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The antigen can be any antigen against which an (increased) immune response is to be obtained, or any part or fragment thereof. Preferably, any such part or fragment is such that it *per se* is capable of eliciting an immune response, such as an epitope. However, this is not required: because according to the invention the fragments are directed to the dendritic cells, i.e. with increased specificity or affinity, a part or fragment that would normally be incapable of eliciting an immune response may provide an immune response when used in conjunction with a ligand for the C-type lectins as described herein. Also, in general, using an antigen in combination with a ligand for the C-type lectins may increase the potency of the antigen, i.e. provide a higher or stronger immune response per unit of antigen administered. In this way, antigens – including those present in serums or vaccines, but also retroviral vectors encoding a desired antigen – could be administered at a lower dosage and still provide sufficient immune response.

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- Figures 2A – 2D are graphs showing that the antibodies AZN-D1 and AZN-D2 inhibit adhesion of DC to ICAM-3 and recognize an antigen that is specifically expressed by DC.

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- Figures 5A-D show the tissue distribution of DC-SIGN as determined by immunohistochemical analysis of the expression of DC-SIGN in tonsils (A and B) and lymph node sections (C and D) (OMx100).

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- Figure 8 shows that DC SIGN binds to both ICAM-2 as well as ICAM-3 expressing K562 cells.

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To identify DC-SIGN, a preparative immunoprecipitation from a DC lysate with the anti-DC-SIGN antibody AZN-D1 was performed and the 44 kDa protein from the SDS-PAGE gel

was excised. After tryptic digestion, the resulting peptides were extracted from the gel and purified using preparative HPLC. Subsequently, the amino acid sequences of 2 peptides (0.5-1 pmol) were determined using the Edman degradation procedure. Both peptides consisted of 11 amino acid residues (Figure 3B; SEQ ID NOs:5 and 6) and the peptide sequences were used to screen the EMBL database for homology with known proteins. Both peptides proved 100% identical to the deduced amino acid sequence of the human HIV gp120-binding C-type lectin (Curtis et al., 1992). This protein has previously been identified exclusively in placenta as a CD4-independent receptor for the human immunodeficiency virus (HIV) envelope glycoprotein gp120.

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To further investigate the expression of DC-SIGN *in vivo*, immunohistochemical analysis of secondary lymphoid tissues with the anti-DC-SIGN antibodies was performed. These tissues are known to contain dendritic cells. Sections of tonsils and lymph nodes contained DC-SIGN expressing cells, which were predominantly observed in the T cell area of both tonsils and lymph nodes (Figures 5A-D). Figures 5A-D show the tissue distribution of DC-SIGN: Immunohistochemical analysis of the expression of DC-SIGN in tonsils and lymph node sections (OMx100). Sections were fixed with acetone and the nuclear staining was performed with Hematein. Staining of DC-SIGN was performed with AZN-D1. The germinal center (GC), T-(T) and B-cell (B) areas are depicted.

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Consistent with the distribution and morphology of dendritic cells, DC-SIGN expressing cells are not detected in the germinal centers and the mantle zone (MZ) of the lymphoid tissues (Figures 5A and 5C). Staining of serial sections for CD3 and CD14 confirmed that the DC-SIGN expressing cells are distinct from T cells and monocytes (data not shown) as was also demonstrated by both flowcytometric analysis and RT-PCR of these cells (Table 1).

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To demonstrate that DC bind to ICAM-3 expressing transfectants in a DC-SIGN dependent manner, the capacity of the leukemic cell line K562 transfected with the cDNA

encoding ICAM-3 (K562-ICAM-3) to bind to DC was investigated. As shown in Figure 6A, DC cluster with K562-ICAM-3 in a DC-SIGN dependent manner, since the interaction can be blocked by anti-DC-SIGN antibodies. No clustering was observed between DC and K562 demonstrating that ICAM-3 is the ligand for DC-SIGN. Figures 6A-D show that DC-SIGN mediated adhesion of DC to ICAM-3 is involved in the DC-T-lymphocyte interaction:

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To test this it was investigated whether clustering of DC to T cells is mediated by DC-SIGN and whether this interaction is also transient. DC were incubated with resting allogeneic T cells (DC:T cell, 1:20) and the DC-T cell clustering was determined. As shown in Figure 6C, the clustering of DC with T cells is transient and reaches a maximum after 20 min (32%). Furthermore, the DC-T cell interaction can be inhibited (approximately 50%) by anti-DC-SIGN antibodies suggesting that the DC-T cell clustering is also mediated by other surface receptors. Thus, the DC-T cell clustering is indeed transient and partly mediated by DC-SIGN/ICAM-3 interactions. Similarly, Figure 8 shows that DC-SIGN binds not only with K562 cells expressing cDNA encoding ICAM-3, but also to K562 cells expressing cDNA encoding ICAM-2, and that said binding can be inhibited by both mannan as well as anti DC-SIGN antibodies.

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As DC-SIGN binding to ICAM-3 is important for the clustering of DC with T cells, the role of DC-SIGN in DC induced T cell proliferation was also investigated. Resting T lymphocytes were stimulated with allogeneic DC in the presence or absence of the blocking anti-DC-SIGN antibodies. As shown in Figure 6D, the anti-DC-SIGN antibodies AZN-D1 and AZN-D2 both inhibited the T-lymphocyte proliferation for more than 75%. Similarly, antibodies against the costimulatory molecule LFA-3, which binds to CD2 on T cells and is also known to mediate T cell proliferation, inhibit T cell proliferation. A combination of anti-LFA-3 and anti-DC-SIGN antibodies completely inhibits T-cell proliferation (Figure 6D).

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Total RNA was isolated by an acidic guanidinium isothiocyanate-phenol-chloroform procedure (Chomczynski and Sacchi, Anal Biochem 162(1), 156-9, 1987). The cDNA encoding

the placenta gp120 binding C-type lectin was amplified by RT-PCR on total RNA from DC. PCR primers were based on the nucleotide sequence of the placenta gp120 binding C-type lectin (accession no. M98457, (Curtis et al., 1992)) and the nucleotide sequences (5' to 3') are as follows: XF29, AGAGTGGGGTGACATGAGTG (SEQ ID NO:3); XR1265, GAAGTTCTGCTACGCAGGAG (SEQ ID NO:4). The PCR fragment was cloned into the pGEM-T vector (Promega, Madison, WI) and sequenced. The nucleotide sequence of the cloned cDNA was identical to that of placenta gp120 binding C-type lectin (Curtis et al., 1992). The cDNA was subsequently cloned into the eukaryotic expression vector pRc/CMV (pRc/CMV-DC-SIGN) and COS7 cells were transient transfected with pRc/CMV-DC-SIGN using the DEAE dextran method (Seed and Aruffo, Proc. Natl. Acad. Sci. U.S.A. *84*, 3365-3369, 1987).